EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Autoplate 4000 Automated Spiral Plater

SOP Number: QC-20-01

Date Revised: 08-22-02

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Date Issued: ___/___/___

Withdrawn By: _____ Date: ___/___/___

Controlled Copy No.: _____

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## 1.0 SCOPE AND APPLICATION:

1.1 This protocol describes the method for calibrating and operating the Spiral Biotech Autoplate 4000 automated Spiral Plater.

2.0 DEFINITIONS: None

3.0 HEALTH AND SAFETY:

3.1 Laboratory personnel should follow biosafety procedures appropriate for the organism being confirmed as outlined in SOP MB-01, Lab Biosafety.

4.0 CAUTIONS:

4.1 Uniform, level, bubble-free, and dry agar plates are necessary. For 100 mm plates, dispense approximately 20 mL of tempered agar per plate; for 150 mm plates, dispense approximately 45 mL.

4.2 Operations can be aborted for cause (i.e., the pertri dish cover is not removed and the plating cycle begins) by turning the POWER off or pressing the RESET button.

4.3 If the stylus support tube becomes contaminated through immersion in sample liquid, wipe with 70% ethanol and let dry before proceeding.

5.0 INTERFERENCES: None

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Spiral Biotech Autoplate 4000 Automated Spiral Plater (Serial Number AP4A187)

7.2 Spiral Biotech Vacuum Source Model 200A (serial Number 358)

7.3 Whatman Inlet Vacu-Guard, Model L#529

7.4 U.S. Gauge Vacuum Gauge, 0-30 inches (Hg) pressure

7.5 Spiral Biotech Polypropylene Reservoirs: Water 1, Water 2, Disinfectant

7.6 Lucite cup holder.

7.7 Disinfectant: Sodium hypochlorite solution equivalent to full strength commercial bleach solution (approximately 5%)

7.8 Dye Solution: 0.7% powdered crystal violet dissolved in water

7.9 Spiral Autoplate Validation Test Fixture

7.10 Spiral Biotech CASBA 4 with Colony Image Analyzer (Serial Number C40399)

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Factory Validation: Prior to shipment, the plunger displacement for Autoplate 4000 was validated for four programmed expulsion rates. A Validation Data Base was included with the Autoplate. The plater was also re-validated on-site by a factory representative. The plater will be factory serviced, including re-validation, annually. (see ref 15.1)

8.2 Laboratory Validation: When in use, the OPP Microbiology Laboratory will conduct a validation test quarterly using the Spiral Biotech Validation Test Fixture. Validation Procedures are outlined in Section 5.2 of the User's Guide (see ref 15.1).

9.0 SAMPLE HANDLING AND STORAGE: Not applicable

10.0 PROCEDURE AND ANALYSIS:

10.1 Equipment Setup and System Check

10.1.1 Remove cover from autoplater.

10.1.2 Place three reservoirs, with covers on, to the left of the turntable in the following order, left to right: Water #2,

Water #1, Disinfectant. The liquid level band should be to the rear.

10.1.3 Remove covers, one at a time, and fill the water station reservoirs with sterile water and the disinfectant reservoir with 5% sodium hypochlorite solution to the top of the liquid bands. Replace covers, leaving approximately a 1.5 inches (4 cm) gap at the rear.

10.1.4 Turn on vacuum source. Pump should reach at least 15" Hg. Normal operating range is 15-20" Hg.

10.1.5 Turn on Spiral Plater. Plater is initialized and ready to operate when the “Ready” light is illuminated.

10.1.6 Run POWER CLEAN cycle. Press POWER CLEAN then CLEAN. Liquid should be evident moving through the vacuum tubing and the vacuum pump should come on during the cleaning cycle. Liquid should not be expelled from the stylus at the second water station. If problems are encountered, consult User Guide Section 7.2 (see ref. 15.2).

10.1.7 Verify performance of the plater on the day of, and prior to plating any samples.

10.1.7.1 Place a 15 x 100 mm trypticase soy agar(TSA) plate on the turntable within the spring retaining clamps. Make sure the plate is seated completely. Remove cover.

10.1.7.2 Confirm Stylus Start Point. Press “100 mm” then TEST to lower the stylus into its start position. Stylus should rest at the intersections of the turntable radial line (9 o’clock position) and 13 mm radial circle etched on the turntable. This is illustrated on page 11 of Reference 15.2. Tolerances are ∓ 3 mm from front to back and ∓ 0.5 mm left to right. If adjustments are necessary, consult Section 5.1 of the User Guide. Press TEST again to return

stylus to its rest position.

10.1.7.3 Check centering of plate (see ref. 15.2). The distance from the scribed line to the outer edge of the plate should be constant. Adjust, if necessary, using thumbscrews (Section 2.3 of ref. 15.2).

10.1.7.4 Confirm settings: Deposition is exponential 50 µl, dish is 100 mm, and sample settings for AUTO is MIN.

10.1.7.5 Run POWER CLEAN cycle.

10.1.7.6 Fill syringe with 0.7% crystal violet solution: In AUTO mode place approximately 3 mL in sample cup (to top edge of lucite holder) in back left corner position of cup holder. In MANUAL, move stylus carriage to extreme right position by depressing STYLUS right arrow (▷), then depressing STYLUS down arrow. In AUTO mode press FILL; in manual mode insert stylus one-half the length of its tip into a container of the dye solution and press MIN.

10.1.7.7 Run Dye Plate. Press PLATE. The stylus will move to the plate and deposit the sample.

10.1.7.8 Check quality of dye plate pattern. It should be continuous with the stronger color near the center; color should gradually fade with distance from origin. If pattern is uneven, check stylus alignment (User Section 5.1) or Troubleshooting (Section 7.5). Confirm by running additional dye pattern.

10.1.7.9 Check alignment of spiral pattern. This should conform to the pattern on the counting grid. If not, check to see that the starting point is set correctly.

10.1.7.10 Run POWER CLEAN cycle.

10.2 Plate Selection, Handling and Labeling

10.2.1 Maximum colony-forming units (CFUs) per plate: 100 mm plates=4.0 x $10^5$ CFU/mL; 150 mm plates=5.0 x $10^6$ cfu/mL

10.2.2 Agar in plates should be uniform, level and free of air bubbles, contaminants, or areas of dehydration.

10.2.3 Plates should be dried inverted for least 24 hours at room temperature or dried with lids open in a biological safety cabinet for at least 15 minutes before use to remove water droplets.

10.2.4 Plates should be labeled on the side of the dish only. Include a vertical line (stylus start mark) and sample identification

10.2.5 Placement of plates on turntable. Plates 100 mm in diameter are secured by an adapter with spring retaining clips. This adapter must be removed to accommodate 150 mm plates. The latter are held in place by three adjustable sliding clips which should be set to both hold plate firmly and centered.

10.3 Plater Deposition Settings

10.3.1 For routine exponential plating to determine inoculum titre, select SLO/50 deposition as the standard default by pressing SHIFT, then SLO/50. The green light at the top left of this button should blink. To cancel, press SHIFT again.

10.3.2 Other settings. Consult User Guide Section 1.3, Keypad Layout and Functions, and 3.4, Deposition Mode Selection (see ref. 15.2).

10.4 Sample preparation

10.4.1 Consult SOP MB-04, Carrier Counts, for preparing dilutions.

10.4.2 Samples may be drawn from either culture tubes or Spiral Plater sample cups. Samples in the cups should be approximately 3 mL, or to the juncture of the cup and upper surface of the lucite holder.

10.5 Plating samples

10.5.1 Run POWER CLEAN cycle.

10.5.2 Remove lid from agar plate.

10.5.3 Automatic Intake: Select MIN (for a single plate) or MAX (enough for 4-5 replicates) and press FILL.

10.5.4 Manual Intake: Press right stylus button to move carriage to extreme right position, then the down stylus button to drop stylus for sample intake. Lower stylus into test tube so that Teflon tip is inserted halfway to the stylus holder in the test solution. Press MIN (for a single replicate) or MAX (enough for 4-5 replicates) to fill.

10.5.5 Press PLATE.

10.5.6 Close agar plate lid as soon as stylus withdraws from turntable.

10.5.7 Set agar plate on level surface and allow liquid to be absorbed for 30 minutes before inverting and placing in an incubator.

10.5.8 Run POWER CLEAN cycle before running additional samples.

10.6 Shutdown procedure

10.6.1 Run power clean cycle. Leave the system full of water when not in use to prevent drying out of the valve tubing.

10.6.2 Remove and empty sterile water and disinfectant reservoirs.

10.6.3 Turn off plater.

10.6.4 Turn off vacuum source.

10.6.5 Wipe panel surface and plating surface with 70% ethanol.

10.6.6 Replace dust cover.

10.6.7 Disinfect reservoirs with 70% ethanol and sterile water in BSC and store closed until ready to use again.

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 Manual Enumeration: Follow procedure described in Section 4 of the Spiral Plater User Guide (see ref 15.2).

11.2 Automated Enumeration: Follow procedure described in CASBA™ 4 with CIA-BEN Software User Guide (see ref 15.1).

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink. Information on Quality Control will be recorded on the Quality Control Record Log Sheet (see 16.1). Printouts generated by the CASBA™, completed forms, and reports are archived in notebooks kept in locked file cabinets in the file room D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule contained on SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 If laboratory validation and calibration results are not within stated tolerance limits, the machine should not be used for sample plating until it is serviced and recertified.

14.2 If other problems arise with the operation of the Autoplate, refer to Section 7 of the user manual (see ref. 15.2), then call the service representative if the problem cannot be solved.

15.0 REFERENCES:

15.1 Spiral Biotech, Inc. 1998. CASBA™ with CIA-BEN Software. User Guide Version 4-27-98.

15.2 Spiral Biotech, Inc. 1999. Autoplate 4000™ Automated Spiral Plater User Guide, Version 4.3 (01/01/99).

16.0 FORMS AND DATA SHEETS:

16.1 Quality Control Record Log Sheet

# Quality Control Record Log Sheet
OPP Microbiology Laboratory

| QUALITY CONTROL RECORD LOG: Autoplate 4000™ | | | | | |
|---|---|---|---|---|---|
| Date/Init. | Activity F,L,P* | Spiral Pattern | Spiral Position | Delivery Rate (F and L Only) | |
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* **F = F**actory Validation, **L = L**aboratory Validation, **P = P**erformance Verification (before each use)